FLUORESCENT TREPONEMAL ANTIBODY STUDIES*

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Fluorescent antibody methods were originally introduced by Coons and associates in 1942. Included in this first publication was a procedure for antibody-fluorescein conjugation and a description of fluorescent reactions obtained by means of ultraviolet light microscopy. This communication, and later ones by other authors, indicated that fluorescent antibody procedures were practical for rapid and direct identification of infectious agents, and might be applied in a variety of fields.

Fluorescent antibody reactions may be obtained in either of two ways. The direct reaction is most frequently employed for pathogen identification and consists of preparing a highly specific antiserum in an appropriate animal and conjugating the gamma globulin with fluorescein. The value of the finished product or conjugate is judged by its ability to react with, or stain, a specific pathogen and no others.

The indirect reaction in contrast to the direct procedure is performed in two stages. First, the pathogen is treated with a specific unlabeled antiserum (example: rabbit serum); and then after washing thoroughly to remove unreacted serum, fluorescein labeled goat antirabbit globulin is applied to detect the presence of rabbit antibody. The Fluorescent Treponemal Antibody (FTA) test is an example of the indirect reaction, and as employed, is used to detect human treponemal antibody. Since the pathogen (*Treponema pallidum*) is a known quantity, the procedure has been modified to detect the unknown quantity (human treponemal antibody or human globulin).

The FTA test is performed in the following manner. First, *Treponema pallidum* is extracted from rabbits in the same manner as is used in the Treponema Pallidum Immobilization (TPI) test. The intact treponemes are dried on microscope slides, and after fixing with acetone, are exposed to the human test serums. Slides are gently rotated at 37° C. to facilitate antibody coupling, and are then thoroughly washed with saline to remove excess serum. Fluorescein labeled anti-human globulin, the reaction indicator, is then added to the smear. Treponemes which have coupled with human antibody will, in turn, couple with the fluorescein conjugate. The reaction can then be visualized with an ultraviolet light microscope. A smear treated with a strongly reactive serum will reveal brilliantly fluorescent treponemes. A smear treated with a serum containing smaller amounts of antibody will demonstrate treponemes with less brilliance or none at all.

FTA reactions are illustrated in the following photomicrographs:

Figure 1—*Treponema pallidum* as seen by the usual darkfield using tungsten light.

Figure 2—*Treponema pallidum*, weakly fluorescent as seen by UV light (weakly reactive serum—treponemes with less brilliance would not be seen) and would constitute a nonreactive result.

Figure 3—*Treponema pallidum*, brilliantly fluorescent as seen with UV light—reactive result.

On the basis of extended studies, the antibodies detected by the FTA test appear to be similar or identical to those identified by the well known TPI test. This conclusion is supported by comparative findings in the Serology Evaluation and Research Assembly (SERA) Study conducted in 1957 by the Venereal Disease Branch, CDC. In this work, six TPI procedures, as performed by five laboratories, are compared. The following results illustrate the close agreement of TPI and FTA findings.

In Category A, Presumed Normals—346 specimens

Range TPI's	1.7–5.5% Reactive
FTA	1.5% Reactive

Category B, Diseases Other Than Syphilis—83 specimens

Range TPI's	9–13% Reactive
FTA	7% Reactive

Category C, Untreated Primary Syphilis—130 specimens

Range TPI's	25–50% Reactive
FTA	60% Reactive

Presented at the Twentieth Annual Meeting of The Society for Investigative Dermatology, Inc., Atlantic City, New Jersey, June 7, 1959.

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FIG. 1

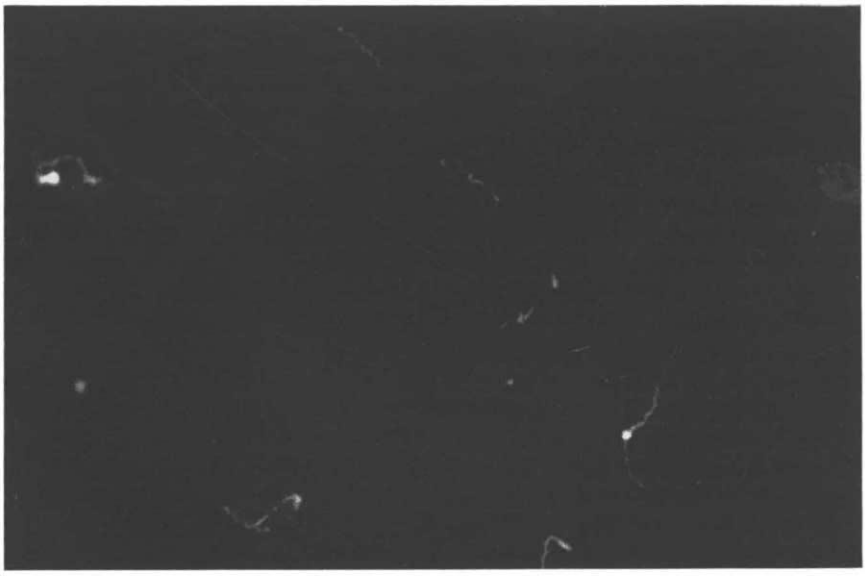

FIG. 2

Category D, Treated Early Syphilis—51 specimens

Range TPI's	27–53% Reactive
FTA	38% Reactive

Category H, Leprosy—29 specimens

Range TPI's	0–3% Reactive
FTA	0% Reactive

The following slides illustrate the relative sensitivity and specificity of various test procedures entered in the SERA Study. Sensitivity as used in these illustrations is based on known syphilis categories consisting of 504 specimens (early, untreated; early, treated; and late, treated). Specificity is based on results obtained with Presumed Normals, and Diseases Other Than Syphilis, consisting of 409 specimens.

Figure 4 shows the distribution of test results. *Circles* indicate *Treponema pallidum* tests, *triangles* are lipoidal tests, *squares* are Reiter treponeme procedures. The large *square* at the

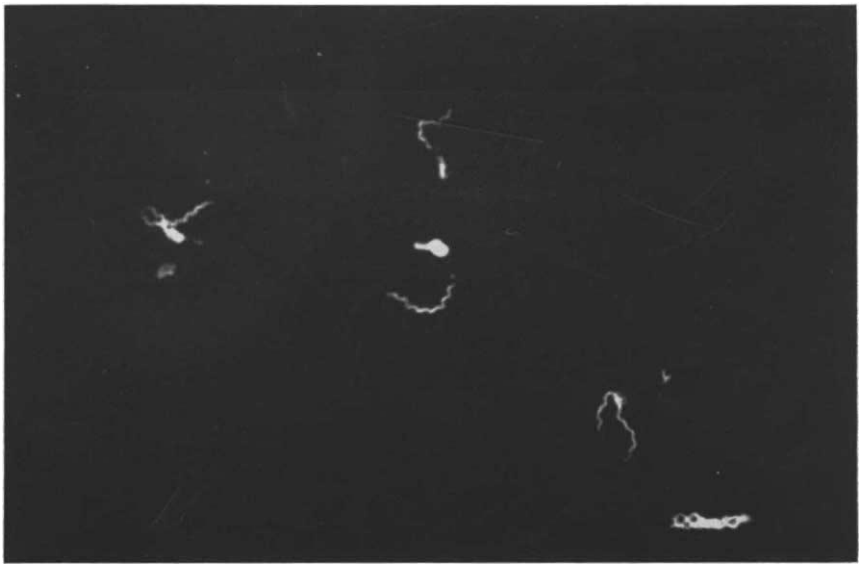

FIG. 3

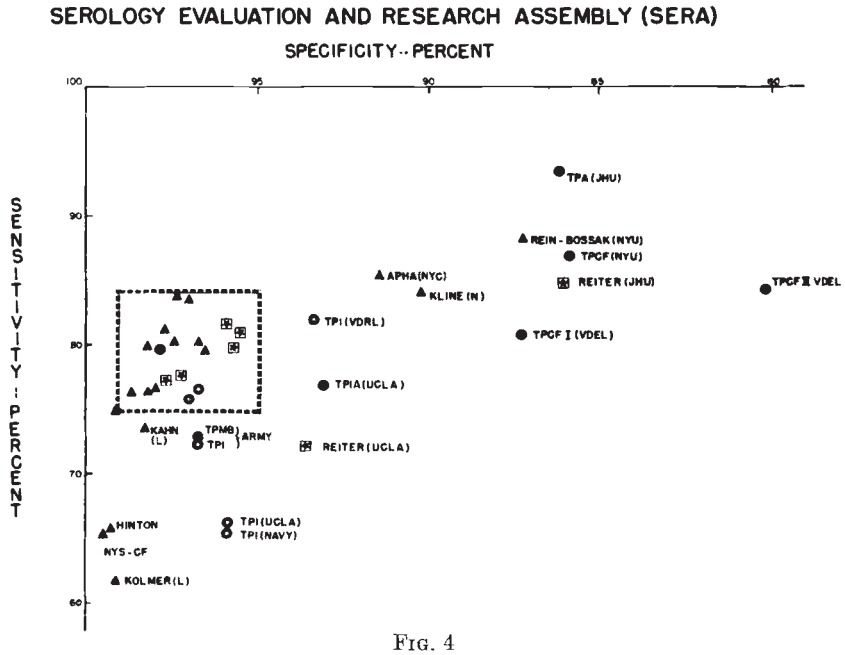

FIG. 4

left encloses those tests with the most desirable results in terms of specificity and sensitivity.

Figure 5 is an enlargement of the left hand square. Test results are identified. Three *Treponema pallidum* tests (TPI, U. Michigan, TPI-200, VDRL), FTA (VDRL), are within the square. Of the three procedures, the FTA test in this analysis is the most favorably located.

Further studies on the mechanism of the FTA test have now been accomplished and may be described briefly as follows:

Lipid antibody produced in rabbits by injection of VDRL antigen, when used in the FTA procedure, reveals that surface antigens of *Treponema pallidum* do not react with this material. On the other hand, contaminating sub-

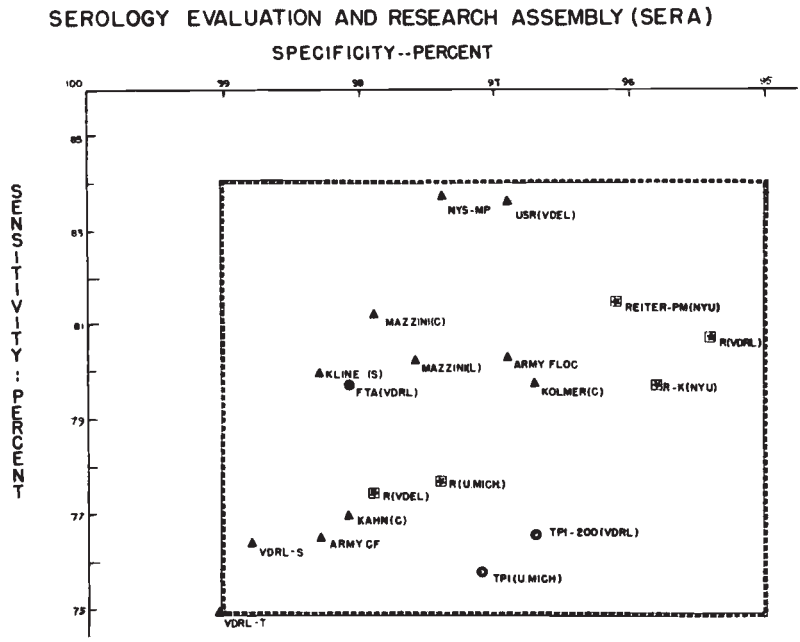

FIG. 5

stances which are found to be present in all treponemal smears obtained from rabbit testes were shown to react with lipid antibody. This finding suggested that normal testicular tissue might serve as a source of nonspecific reactivity. The removal of reactivity to VDRL antigen from rabbit and human sera by absorption with normal testicular tissue has proven this antigenic relationship.

Absorption of reactive serum with VDRL antigen removes the reactivity with several serological tests for syphilis, but leaves reactivity when tested with the FTA procedure and the TPI.

Since Hardy, *et al.*, and other workers have described intact *Treponema pallidum* antigens as being reactive in both lipid and treponemal antibody systems, an investigation of the Hardy antigen in the FTA procedure was undertaken. Smears were prepared in the usual manner using Hardy's antigen and were exposed to rabbit anti-VDRL antigen globulin. Subsequent treatment with fluorescein labeled goat antirabbit indicator confirmed the lipid antigenic activity of *Treponema pallidum* prepared by the Hardy method. On the basis of this experiment and those previously described, it would appear that reactive lipids are not a part of the normal antigenic surface constituents of intact *Treponema pallidum* as used in the TPI test. The source of this material, as previously indicated, is probably normal testicular antigen which has been placed on the treponeme surfaces during the extraction process.

CONCLUSIONS

The FTA test is basically a simple procedure. Problems arising from nonspecificity are most likely concerned with the treponemes (lipid antigens), and as a result, carefully tested antigens should be selected. That is, each new batch of antigen should be checked for lipid contamination. This may be done by checking with anti-lipid serums. Also, tests should be made for sensitization of the treponemes by rabbit antibodies. Once an antigen has been found satisfactory, it may be stored in several ways, such as, refrigeration at 5° C., freezing at −40° C., and by lyophilizing. Fluorescein labeled antihuman and antirabbit globulins are available commercially. The greatest cost appears to be in purchasing fluorescent equipment. However, with the increasing applications of fluorescent antibody technics, the need for such expenditure is justified.

DISCUSSION

DR. MARION B. SULZBERGER (New York, N. Y.): I would like to just discuss the point about the absorption of the anti-lipoidal antibodies by the testicular material. I would like to ask Dr. Freeman and Dr. Deacon whether they have tried the fluorescent antibody technic against the culture Reiter treponemes, which might not carry any of these organ materials since they have been cultured on thioglycollate medium. If the authors have done so, what have the results been?

DR. IRA L. SCHAMBERG (Elkins Park, Pa.): As a syphilophile for a good many years now, I am gratified to hear this group of three papers on syphilis. They bespeak our continued interest in syphilology, a field to which our professional forbears contributed much of which we are proud. I am especially proud to recall my own father's clinical and laboratory studies in syphilis. But there are signs in recent years that we are losing our interest in this fascinating disease. Only one of the papers presented today was by a member of our dermatologic fraternity. Fewer and fewer of us have a major interest in syphilis. Hence, the death of Dr. Charles Rein a few years ago was not only a tragedy to those of us who loved him, but left an unfilled void in the field to which he gave so much.

We hear the plaint that there are not enough syphilitic patients in teaching hospitals to train students. Bruce Webster of New York gave a most stimulating talk at the recent Symposium on Venereal Diseases in Baltimore on this subject. He pointed out that the crying need is for somebody interested in teaching syphilis. Such a man will find plenty of material in any hospital today. In a community hospital in an upper middle class area of Philadelphia, with which I am connected, I saw within the past six weeks one patient with advanced tabes dorsalis, one with a wide open syphilitic aortic regurgitation, one with a syphilitic aortic aneurysm filling half of his chest as well as, of course, a number of serologic problems.

I am happy to have heard these papers. I hope we will hear more of them as the years go on. I hope that dermatologists will continue to be syphilologists.

MISS ELIZABETH M. FREEMAN (in closing): Thank you for the discussion. In answer to Dr. Sulzberger's question, the use of the Reiter treponeme in the FTA test has not given specific reactions in our hands. This is probably due to lipid antigen on its surface. The availability of fluorescein isothiocyanate, and the brilliance obtained when using this compound, offers the opportunity to label specific sera to prove the relationship of the Reiter and the Nichol strain. We feel these findings may be very interesting.